IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Alexander Steinbüchel et al.

Serial No.: To be Assigned

Filed: Herewith

For: METHODS FOR PRODUCING POLY(HYDROXY) FATTY ACIDS IN BACTERIA (As amended)

Group Art Unit: To be Assigned

Examiner: To be Assigned

Atty. Dkt. No.: 11899.0152.DVUS01
(MOBT:152-2/KAM)

# PRELIMINARY AMENDMENT

CERTIFICATE OF EXPRESS MAILING

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I hereby certify that this paper or fee is being deposited with the United States Postal Service "EXPRESS MAIL POST OFFICE TO ADDRESSEE" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to: Commissioner for Patents, Washington, DC 20231.

Maria L. Alvarez
Signature

Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend this application as follows:

## AMENDMENTS

**In The Specification**

Please, delete the original title and insert a title to read: --METHODS FOR PRODUCING POLY(HYDROXY) FATTY ACIDS IN BACTERIA--

At page 2, delete the first paragraph, and insert the following:

--This is a divisional of co-pending application Serial No. 09/420,119, filed October 18, 1999, which is a divisional application of Serial No. 08/809,286 filed July 3, 1997 now issued (US Patent No. 6,011,144), which is a § 371 National Stage Application of

PCT/DE95/01279, filed September 15, 1995, which claimed priority to German application P 4433 134.7 filed September 16, 1994. - -

At page 33, please insert the Sequence Listing provided herewith.

**In the Claims**

Please cancel claims 19-22, and 33-36, without prejudice.

Please amend the following claims:

3. Process in accordance with Claim 1 [~~or 2~~], characterized by the feature that one also adds to the bacterial culture at least one additional carbon source which promotes growth, whereby the carbon source is selected from the group comprising:

   citric acid, octanoic acid and gluconic acid; their salts, esters and lactones; hexoses, especially glucose and fructose; as well as their mixtures.

4. Process in accordance with **claim** 1 [~~one of the Claims through 3~~], characterized by the feature that the process is carried out in the form of a batch process, a fed-batch process, a two-step process or a continuous flow process.

5. Process in accordance with **claim 1** [~~one of the Claims 1 through 4~~], characterized by the feature that the poly(hydroxy fatty acid) is obtained in a concentration of approximately 15 to 70% by weight or, especially approximately 15 to 50% by weight or, preferably, approximately 40% by weight based on the dry mass of the bacterial cells.

6. Process in accordance with **claim 1** [~~one of the Claims 1 through 5~~], characterized by the feature that the poly(hydroxy fatty acids) are obtained in the form of copolyesters with at least two or, preferably, three subunits.

7. Process in accordance with **claim 1** [~~one of the Claims 1 through 6~~], characterized by the feature that the recombinant bacteria are cultivated at cell densities of up to 100 g of dry cellular mass per liter of bacterial nutrient medium.

8. Process in accordance with **claim 1** [~~one of the Claims 1 through 7~~], characterized by the feature that one offers the substrate carbon source in excess.

12. Process in accordance with **claim 1** [~~one of the Claims 1 through 11~~], characterized by the feature that cultivation takes place for approximately 24 h to 96 h or, especially, for approximately 36 h to 72 h or, preferably, for approximately 48 h to 72 h.

13. Process in accordance with **claim 1** [~~one of the Claims 1 through 12~~], characterized by the feature that the recombinant bacteria are cultivated under conditions of deficiency, preferably under conditions of a deficiency of nitrogen, magnesium or phosphate.

14. Process in accordance with **claim 1** [~~one of the Claims 1 through 13~~], characterized by the feature that the harvested recombinant bacteria are broken open by means of physical and/or chemical and/or biochemical processes in order to obtain the poly(hydroxy fatty acids) that have been produced bio-technically.

23. Poly(hydroxy fatty acid) which is obtainable using a process in accordance with **claim 1** [~~one of the Claims 1 through 18~~].

## REMARKS

The active claims in this case are claims 1-18, and 24-32. Claims 1-18, and 24-32 correspond to original non-elected group I in the original parent application USSN 08/809,286. No new matter is introduced in claims 1-18, and 24-32. For the convenience of the Examiner, a list of pending claims is attached at the end of this document.

The specification has been amended to recite the relationship with the parent case, namely that this application is a divisional of co-pending application Serial No. 09/420,119, filed October 18, 1999, which is a divisional application of Serial No. 08/809,286 filed July 3, 1997 now issued (US Patent No. 6,011,144), which is a § 371 National Stage Application of PCT/DE95/01279, filed September 15, 1995, which claimed priority to German application P 4433 134.7 filed September 16, 1994.

Further, applicants request acknowledgement of their claim to priority.

It is believed that no fee is due; however, should any fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason, the Commissioner is authorized to deduct said fees from Howrey Simon Arnold & White, LLP Deposit Account No. 01-2508/11899.0152.DVUS01/KAM.

Respectfully submitted,

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**PENDING CLAIMS FOR MOBT:152-2**

1. Process for the preparation of poly(hydroxy fatty acids) with at least one subunit by means of recombinant bacteria which contain and express at least one fragment of the gene of poly(hydroxy fatty acid) synthase from *Thiocapsa pfennigii* and which are selected from the group comprising:

   *Pseudomonas putida* GPpl04 (pHP1014::E156), *Alcaligenes eutrophus* PHB 4 (pHP1014::EIS6), *Pseudomonas putida* GPpl04 (pHP1014::B28+) [DSM # 9417] and *Alcaligenes eutrophus* PHB 4 (pHP1014:B28+) [DSM # 9418], whereby the bacteria are cultivated in a mineral medium under aerobic conditions, whereby

   - one offers the bacteria at least one substrate carbon source which is selected from the group consisting of:

     levulinic acid, salts of levulinic acid, esters of levulinic acid, lactones of levulinic acid, substituted levulinic acid or, as the case may be, its derivatives; 5-hydroxyhexanoic acid, its salts, esters and lactones; 4-hydroxyheptanoic acid, its salts, esters and lactones; 4-hydroxyoctanoic acid, its salts, esters and lactones; their halogenated derivatives as well as their mixtures;
   - one incubates the bacteria for a certain time with the carbon source; and
   - one isolates the poly(hydroxy fatty acid) polymers that have been synthesized by the bacteria.

2. Process in accordance with Claim 1, characterized by the feature that the bacteria are pre-cultivated in a complex medium.

4. (Amended) Process in accordance with claim 1, characterized by the feature that one also adds to the bacterial culture at least one additional carbon source which promotes growth, whereby the carbon source is selected from the group comprising:

   citric acid, octanoic acid and gluconic acid; their salts, esters and lactones; hexoses, especially glucose and fructose; as well as their mixtures.

4. (Amended) Process in accordance with claim 1, characterized by the feature that the process is carried out in the form of a batch process, a fed-batch process, a two-step process or a continuous flow process.

5. (Amended) Process in accordance with claim 1, characterized by the feature that the poly(hydroxy fatty acid) is obtained in a concentration of approximately 15 to 70% by weight or, especially approximately 15 to 50% by weight or, preferably, approximately 40% by weight based on the dry mass of the bacterial cells.

6. (Amended) Process in accordance with claim 1, characterized by the feature that the poly(hydroxy fatty acids) are obtained in the form of copolyesters with at least two or, preferably, three subunits.

7. (Amended) Process in accordance with claim 1, characterized by the feature that the recombinant bacteria are cultivated at cell densities of up to 100 g of dry cellular mass per liter of bacterial nutrient medium.

8. (Amended) Process in accordance with claims 1, characterized by the feature that one offers the substrate carbon source in excess.

9. Process in accordance with Claim 8, characterized by the feature that one uses the substrate carbon source at a concentration of approximately 0.1 to 5% by weight.

10. Process in accordance with Claim 9, characterized by the feature that one increases the concentration of the substrate carbon source in the culture medium in steps, optionally with pre-cultivation in the presence of an additional carbon source which does not serve as a substrate.

11. Process in accordance with Claim 10, characterized by the feature that, in each case, one adds approximately 0.5% (weight/volume) of neutralized substrate carbon source after approximately 12 h and 24 h at approximately 27°C to 35°C or, preferably, at approximately 30°C.

12. (Amended) Process in accordance with claim 1, characterized by the feature that cultivation takes place for approximately 24 h to 96 h or, especially, for approximately 36 h to 72 h or, preferably, for approximately 48 h to 72 h.

13. (Amended) Process in accordance with claim 1, characterized by the feature that the recombinant bacteria are cultivated under conditions of deficiency, preferably under conditions of a deficiency of nitrogen, magnesium or phosphate.

14. (Amended) Process in accordance with claim 1, characterized by the feature that the harvested recombinant bacteria are broken open by means of physical and/or chemical

and/or biochemical processes in order to obtain the poly(hydroxy fatty acids) that have been produced bio-technically.

15. Process in accordance with Claim 14, characterized by the feature that the harvested recombinant bacteria are lyophilized and then extracted with an organic solvent, preferably chloroform or methylene chloride, in order to break open the recombinant bacteria and to obtain the poly(hydroxy fatty acids).

16. Process in accordance with Claim 15, characterized by the feature that the extracted poly(hydroxy fatty acid) product is precipitated by introducing a hydrophilic solvent, especially water or a lower alcohol, preferably ethanol, and the product is obtained in essentially pure form by removing the hydrophilic solvent.

17. Process in accordance with Claim 14, characterized by the feature that the harvested recombinant bacteria are broken open by means of detergents and/or a lytic enzyme cocktail as a result of which the bacterial cell grana, which contain the poly(hydroxy fatty acid), sediment to the bottom of the bio-reactor and are collected from there in order to be processed further.

18. Process in accordance with Claim 17, characterized by the feature that the lytic enzyme cocktail contains enzymes which are selected from the group which comprises:

lysozyme; proteases; other hydrolytic enzymes; as well as their mixtures.

23. (Amended) Poly(hydroxy fatty acid) which is obtainable using a process in accordance with claim 1.

24. Poly(hydroxy fatty acid) in accordance with Claim 23, characterized by the feature that it contains groups of subunits which are selected from the group which comprises:

(A) 3-hydroxybutyric acid, 3-hydroxyvaleric acid and 4-hydroxy-valeric acid;

(B) 3-hydroxybutyric acid, 3-hydroxyvaleric acid, 4-hydroxy-valeric acid, 3-hydroxyhexanoic acid and 3-hydroxyoctanoic acid;

(C) 3-hydroxybutyric acid, 3-hydroxyhexanoic acid, 5-hydroxy-hexanoic acid and 3-hydroxyoctanoic acid;

(D) 3-hydroxybutyric acid, 3-hydroxyvaleric acid, 3-hydroxy-hexanoic acid, 3-hydroxyheptanoic acid, 4-hydroxyheptanoic acid and 3-hydroxyoctanoic acid;

(E) 3-hydroxybutyric acid, 3-hydroxyhexanoic acid, 3-hydroxy-octanoic acid and 4-hydroxyoctanoic acid;

(F) 3-hydroxybutyric acid, 3-hydroxyhexanoic acid and 5-hydroxy-hexanoic acid;

(G) 3-hydroxybutyric acid, 3-hydroxyvaleric acid, 3-hydroxy-heptanoic acid and 4-hydroxyheptanoic acid;

(H) 3-hydroxybutyric acid, 3-hydroxyvaleric acid, 3-hydroxy-hexanoic acid, 3-hydroxyoctanoic acid and 4-hydroxyoctanoic acid;

(I) 3-hydroxybutyric acid, 3-hydroxyhexanoic acid and 4-hydroxy-hexanoic acid; and

(J) 3-hydroxybutyric acid and 5-hydroxyhexanoic acid.

25. Poly(hydroxy fatty acid) in accordance with Claim 24, characterized by the feature that the poly(hydroxy fatty acid) with the subgroup unit (A) has the following quantitative composition:

approximately 35 mol% to 65 mol% of 3-hydroxybutyric acid;

approximately 30 mol% to 50 mol% of 3-hydroxyvaleric acid; and

approximately 5 mol% to 20 mol% of 4-hydroxyvaleric acid.

26. Poly(hydroxy fatty acid) in accordance with Claim 24, characterized by the feature that the poly(hydroxy fatty acid) with the subgroup unit (B) has the following quantitative composition:

approximately 10 mol% to 15 mol% of 3-hydroxybutyric acid;

approximately 40 mol% to 60 mol% of 3-hydroxyvaleric acid;

approximately 10 mol% to 20 mol% of 4-hydroxyvaleric acid;

approximately 5 mol% to 15 mol% of 3-hydroxyhexanoic acid; and

approximately 2 mol% to 10 mol% of 3-hydroxyoctanoic acid.

27. Poly(hydroxy fatty acid) in accordance with Claim 24, characterized by the feature that the poly(hydroxy fatty acid) with the subgroup unit (C) has the following quantitative composition:

approximately 60 mol% to 80 mol% of 3-hydroxybutyric acid;

approximately 2 mol% to 10 mol% of 3-hydroxyhexanoic acid;

approximately 15 mol% to 30 mol% of 5-hydroxyhexanoic acid; and

approximately 1 mol% to 5 mol% of 3-hydroxyoctanoic acid.

28. Poly(hydroxy fatty acid) in accordance with Claim 24, characterized by the feature that the poly(hydroxy fatty acid) with the subgroup unit (D) has the following quantitative composition:

approximately 30 mol% to 50 mol% of 3-hydroxybutyric acid;
approximately 10 mol% to 30 mol% of 3-hydroxyvaleric acid;
approximately 15 mol% to 35 mol% of 3-hydroxyhexanoic acid;
approximately 1 mol% to 10 mol% of 3-hydroxyheptanoic acid;
approximately 1 mol% to 10 mol% of 4-hydroxyheptanoic acid; and
approximately 1 mol% to 10 mol% of 3-hydroxyoctanoic acid.

29. Poly(hydroxy fatty acid) in accordance with Claim 24, characterized by the feature that the poly(hydroxy fatty acid) with the subgroup unit (E) has the following quantitative composition:

approximately 65 mol% to 85 mol% of 3-hydroxybutyric acid;
approximately 15 mol% to 30 mol% of 3-hydroxyhexanoic acid;
approximately 1 mol% to 5 mol% of 3-hydroxyoctanoic acid; and
approximately 0.5 mol% to 5 mol% of 4-hydroxyoctanoic acid.

30. Poly(hydroxy fatty acid) in accordance with Claim 24, characterized by the feature that the poly(hydroxy fatty acid) with the subgroup unit (F) has the following quantitative composition:

approximately 50 mol% to 80 mol% of 3-hydroxybutyric acid;
approximately 3 mol% to 10 mol% of 3-hydroxyhexanoic acid; and
approximately 10 mol% to 30 mol% of 5-hydroxyhexanoic acid.

31. Poly(hydroxy fatty acid) in accordance with Claim 24, characterized by the feature that the poly(hydroxy fatty acid) with the subgroup unit (G) has the following quantitative composition:

approximately 30 mol% to 80 mol% of 3-hydroxybutyric acid;
approximately 5 mol% to 20 mol% of 3-hydroxyvaleric acid;
approximately 1 mol% to 5 mol% of 3-hydroxyheptanoic acid; and
approximately 3 mol% to 10 mol% of 4-hydroxyheptanoic acid.

32. Poly(hydroxy fatty acid) in accordance with Claim 24, characterized by the feature that the poly(hydroxy fatty acid) with the subgroup unit (H) has the following quantitative composition:

approximately 70 mol% to 90 mol% of 3-hydroxybutyric acid;

approximately 1 mol% to 5 mol% of 3-hydroxyvaleric acid;

approximately 10 mol% to 20 mol% of 3-hydroxyhexanoic acid;

approximately 1 mol% to 5 mol% of 3-hydroxyoctanoic acid; and

approximately 0.5 mol% to 4 mol% of 4-hydroxyoctanoic acid.